ORIGINAL ARTICLE

A marker for acute cholecystitis severity: thiol-disulfide balance and ischemia-modified albumin

Un marcador de la gravedad de la colecistitis aguda: equilibrio de tiol-disulfuro y albúmina modificada por isquemia

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Abstract

Objective: *According to the Tokyo 2018 guidelines, white blood cells (WBCs) are the only markers used in the staging of acute cholecystitis. We aimed to investigate the role of thiol-disulfide and ischemia-modified albumin (IMA), which are used in the diagnosis of inflammatory diseases, in the diagnosis, and severity of acute cholecystitis.* **Method:** *A total of 108 patients hospitalized with acute cholecystitis and 42 healthy volunteers were included in the study. Plasma total thiol (TT), native thiol (NT), and disulfide levels were measured and IMA was calculated using disulfide/native, disulfide/total, and native/TT ratios.* **Results:** *Significant differences were found in both inflammatory and antioxidant markers, age, and symptom duration between disease stages (Stages I, II, and III) and control group ($p < 0.001$). Age and symptom duration were negatively correlated with antioxidant parameters (albumin, NT, and TT) ($r = -0.321$, $p < 0.00$). C-reactive protein and WBC correlated negatively with albumin and antioxidant parameters and positively with disulfide ($r = 0.776$, $p < 0.001$; $r = 0.358$, $p < 0.001$),* **Conclusions:** *The oxidative stress markers in our study can be used to assist radiologic examinations in determining the severity of acute cholecystitis.*

Keywords: *Acute cholecystitis. Ischemia-modified albumin. Tokyo 2018 Guidelines. Thiol/disulfide.*

Resumen

Objetivo: *Según las directrices de Tokio 2018, los glóbulos blancos son los únicos marcadores utilizados en la estadificación de la colecistitis aguda. Nos propusimos investigar el papel del tiol-disulfuro y la albúmina modificada por isquemia, que se utilizan para el diagnóstico de enfermedades inflamatorias, en el diagnóstico y la gravedad de la colecistitis aguda.* **Método:** *Se midieron los niveles totales de tiol, tiol nativo y disulfuro, y de albúmina modificada por isquemia, en plasma de 108 pacientes con colecistitis aguda y 42 voluntarios sanos.* **Resultados:** *Se encontraron diferencias significativas entre los estadios de la enfermedad (I, II y III) y el grupo control en términos de marcadores inflamatorios y antioxidantes, edad y duración de los síntomas ($p < 0.001$). La edad y la duración de los síntomas se correlacionaron negativamente con los parámetros antioxidantes (albúmina, tiol nativo, tiol total) ($r = -0.321$; $p < 0.00$). La proteína C reactiva y los glóbulos blancos se correlacionaron negativamente con la albúmina y los parámetros antioxidantes, y positivamente con el tiol disulfuro ($r = 0.776$, $p < 0.001$; $r = 0.358$, $p < 0.001$).* **Conclusiones:** *Los marcadores de estrés oxidativo de nuestro estudio pueden utilizarse para apoyar a los exámenes radiológicos en la determinación de la gravedad de la colecistitis aguda.*

Palabras clave: *Colecistitis aguda. Albúmina modificada por isquemia. Directrices de Tokio 2018. Tiol-disulfuro.*

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Introduction

Gallbladder stones are detected in 60% of patients who apply to hospitals due to the right upper quadrant (RUQ) pain, and acute cholecystitis is seen as the main cause of related abdominal pain in approximately 50% of these patients[1]. Worldwide, gallbladder diseases are the most common and costly digestive disorders. Acute cholecystitis results from gallbladder stones permanently blocking the cystic duct in 95% of cases. Obstruction increases intraluminal pressure and gallbladder swelling. Without cystic duct patency, neutrophils penetrate the gallbladder wall, causing mucosal bleeding and necrosis[2].

The community incidence of acute cholecystitis in gallstone patients is unknown; however, 20% are diagnosed. About 20% of acute cholecystitis patients develop gangrenous cholecystitis (GC) and 10% have perforation. The acute-phase reactants white blood cell (WBC) and C-reactive protein (CRP) rise dramatically in these conditions[3].

Historically, the Murphy sign and Charcod's triad were the main exam findings for acute cholecystitis and cholangitis. The Tokyo 2018 Acute Cholecystitis and Cholangitis Treatment Guide (TR18) add new recommendations based on prospective studies. This guide advised field physicians to diagnose and treat probable acute cholecystitis or cholangitis to reduce morbidity and mortality[4].

In the patient with acute cholecystitis, the disease condition, which starts with only inflammation at first, may progress to ischemia and perforation in the gallbladder and then cause sepsis and multiple organ failure[5].

Increased inflammatory mediators in acute inflammatory diseases cause an increase in oxidants such as reactive oxygen groups. Oxidants, which are balanced by various antioxidant systems in the body under physiological conditions, reach levels that exceed the antioxidant system with the severity of inflammation. This situation is called oxidative stress. Thiol groups are antioxidants that take excess electrons from oxidants and form disulfide bonds. The increase in disulfide bonds in an environment where oxidants are abundant suggests that disulfide is a marker of oxidative stress. When oxidative stress decreases, disulfide bonds can be reduced back to thiol and, thus, establish the thiol-disulfide balance[6]. During ischemia, free oxygen radicals alter serum albumin structure and reduce heavy metal binding. Albumin changed by ischemia. Ischemia-modified albumin (IMA) was first used to diagnose emergency myocardial ischemia, according to literature. Recent investigations have connected IMA to infections, malignancies, and trauma[7].

In this study, we aimed to investigate thiol-disulfide balance and IMA levels in patients with acute cholecystitis, an acute inflammatory disease that we believe is caused by oxidative stress. Since these tests can be easily measured together with biochemical parameters and WBC is the only laboratory marker in the staging of acute cholecystitis according to the Tokyo 2018 guidelines, we aimed to determine whether oxidative stress markers can help radiological and clinical methods to determine the stage of the disease before hospitalization.

Method

Study design

In this prospective, multicenter descriptive study, a total of 168 individuals, including 126 patients consulted to general surgery with a prediagnosis of acute cholecystitis from patients admitted to the emergency departments of both centers between April 2019 and April 2020, and 42 adult volunteer patients with no organic pathology during their admission to the general surgery outpatient clinic, were included in the study. A total of 18 patients were excluded from the study because six patients did not meet the study criteria, four patients did not sign the consent form, five patients had hemolyzed blood samples, two patients were pregnant, and one patient had a history of malignancy. The remaining 108 patients were divided into Grade 1, Grade 2, and Grade 3 groups using the Tokyo 2018 acute cholecystitis treatment guideline criteria and routinely used WBC count, international normalized ratio (INR), CRP values, and ultrasound, computed tomography data were obtained from the hospital system. Demographic data of the patients and the control group were recorded prospectively by face-to-face interviews with the patients[4] (Fig. 1).

Patient selection

The study included treatment data for patients diagnosed with acute cholecystitis who were admitted to emergency departments or general surgery outpatient clinics across multiple centers. Eligible patients were those over 18 years old, not pregnant, and without a history of chronic disease or drug use. The exclusion criteria were as follows: patients under 18, pregnant women, individuals with chronic or autoimmune

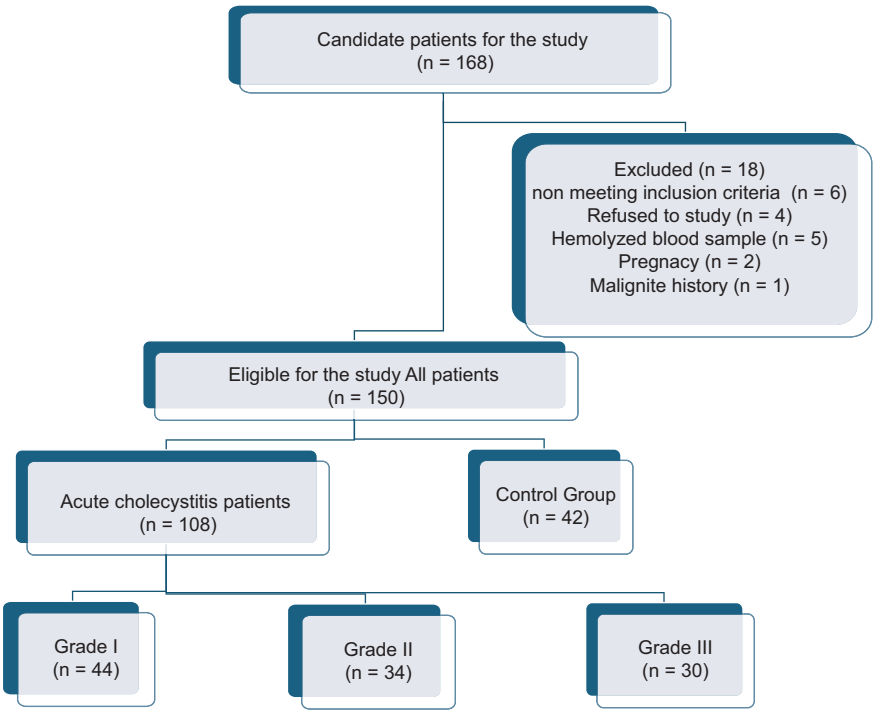

Figure 1. *Flow chart prospective multicenter descriptive study.*

diseases, those who did not sign the consent form, and patients with a history of past or active cancer.

This study was designed to evaluate the interactions between these markers and four different groups based on values obtained from the previous studies in patient groups with other diseases and published in international peer-reviewed journals. The power analysis was based on an effect size of 0.8 and was designed with 90% power and 5% probability of Type I error. The total sample size required for the study was calculated as 28. This sample size was determined to ensure that the study had sufficient statistical power.

Ethical approval

This scientific study Süleyman Demirel University Faculty of Medicine Clinical Research (dated March 05, 2019 decision no. 93) and Isparta Governorship Provincial Health Directorate Research and Development Commission (with document number 59222281-771 dated March 26, 2019) Ethics. It was initiated and carried out after receiving approval from the boards. The study was carried out by the Declaration of Helsinki.

Biochemical analysis

Patients with acute cholecystitis were diagnosed clinically and radiologically. Approximately 5 cc of blood was taken into a routine biochemistry tube before initiating antibiotic therapy, and the blood was centrifuged for 10 min at 3600 rpm at +4°C. The separated serum was placed in an Eppendorf tube and stored at −80°C. Thiol-disulfide and IMA parameters, thiol-disulfide homeostasis parameters, and IMA levels were analyzed. In the analysis of plasma thiol/disulfide homeostasis, Erel and Neşelioğlu the kits of the automatic method developed by him were used[8]. In IMA measurement, Bar-Or et al. the method developed by was used. Since IMA concentrations are not standard, they were given in absorbance units (ABSU)[9]. Native thiol (NT), total thiol (TT), disulphide (D) measurements, D/NT (index 1), D/TT (index 2), and NT/TT (index 3) ratios were evaluated in the biochemistry laboratory regarding the thiol-disulfide balance.

Statistical analysis

SPSS for Windows 25.0 (IBM Corp., Armonk, NY) was used to analyze study patient data. Kolmogorov–Smirnov and Histogram examined continuous variable normality assumptions, skewness and kurtosis coefficients, median, mode, and mean values, and Levene's Test examined variance homogeneities. Descriptive statistics for continuous variables included mean and standard deviation, and categorical variables included frequency (n) and percentage (%). Comparing three or

more levels was done with one-way analysis of variance (ANOVA) for normally distributed data and the Kruskal–Wallis test for non-normal data that *post hoc* tests were used for continuous variables. Bonferroni correction was applied after ANOVA test. Mann–Whitney U-test was performed after Kruskal–Wallis test. X^2/Fisher exact analysis was used for categorical variables, Spearman (Spearman's rho) correlation analysis for continuous variables, and receiver operating characteristic (ROC) analysis for sensitivity and specificity. Relationship categorical variables were tested with Cramer's V. The results were statistically significant when $p < 0.05$.

Results

A total of 150 patients, the study had 42 control participants (28.0%) and 108 patients (72.0%). 40.7% of patients were in Stage I, 31.5% in Stage II, and 27.8% in Stage III. The control group averaged 52.14 years (range: 18-79), while the patient group averaged 56.77 years. The control group had 52.4% female and 47.6% male, while the patient group had 46.3% female and 53.7% male. The participants were split between Isparta City (42.6%) and Suleyman Demirel University Hospital (57.4%). Murphy sign was positive 78.7% of the time and negative 21.3%. About 88.9% of participants had RUQ tenderness, and 25.0% rebounded. RUQ palpable mass was absent in 81.5% and present in 18.5%. Fever was reported by 27.8% of patients and not by 72.2%. The characteristics of the patients are in table 1.

There are significant differences between different grades (Grade I, II, and III) and a control group. Significant differences were observed between groups in terms of WBC, CRP, albumin, IMA, index 1, index 2, index 3, INR, NT, TT, disulfide, age, and symptom duration ($p < 0.001$). WBC, CRP, IMA, index 1, index 2, and INR increased from stage I to stage III, whereas albumin, index 3, NT, and TT decreased numerically. The control group had the lowest mean values for CRP, IMA, index 1, index 2, INR, and symptom duration and the highest mean values for albumin, index 3, NT, and TT compared to the other patient groups (Table 2).

Our correlation analysis revealed complex relationships between age, symptom duration, inflammatory markers, and oxidative stress indicators in acute cholecystitis grading in the patient group. Age has a significant positive correlation with symptom duration ($r = 0.380$, $p < 0.001$), suggesting a role in disease progression. Age was positively correlated with inflammatory markers including CRP and WBC ($r = 0.355$, $p < 0.001$), but negatively correlated with antioxidant markers such as albumin, NT, and TT ($r = -0.321$, $p < 0.001$) (Table 3).

Symptom duration was positively correlated with CRP levels ($r = 0.776$, $p < 0.001$) and WBC count ($r = 0.358$, $p < 0.001$), indicating an inflammatory connection. Conversely, albumin ($r = -0.335$, $p < 0.001$) and antioxidant parameters (NT, TT, and index 3 ratio) showed negative correlations. Positive correlations were found between disulfide ratios (Index 1, index 2) and disease stage ($r = 0.809$, $p < 0.001$) (Table 3).

Table 4 shows the ROC analysis of NT, TT, index 3 ratio, disulfide, index 1 ratio, and IMA for oxidative stress diagnostics. Results showed significant discrimination for each parameter: NT showed an area under the curve (AUC) of 0.858 ($p < 0.001$), with an optimal

Table 1. Sociodemographic and clinical characteristics of the patients included in the study

Variable	n (%)
Group	42 (28.0)
Control group	108 (72.0)
Patient group	
Phase*	
Grade I	44 (40.7)
Grade II	34 (31.5)
Grade III	30 (27.8)
Age	
Control group	52.14 (18-79)
Patient group	56.77 (19-84)
Gender	
Control group	
Female	22 (52.4)
Male	20 (47.6)
Patient group	
Female	50 (46.3)
Male	58 (53.7)
Hospital (site)*	
Isparta City	46 (42.6)
Suleyman Demirel University Hospital	62 (57.4)
Murphy sign*	
Negative	23 (21.3)
Positive	85 (78.7)
Tenderness in the right upper quadrant*	96 (88.9)
Rebound examination finding*	27 (25.00)
SUC palpable mass*	
Negative	88 (81.5)
Positive	20 (18.5)
Fever*	
None	78 (72.2)
There is	30 (27.8)
*Only numbers and percentages in the patient group are given.	30 (27.8)

* For marked variables, only the numbers and percentages in the patient group are given. It does not include the control group.

Table 2. Comparison of BK, CRP, albumin, IMA, index 1, index 2, index 3, INR, native thiol, total thiol, disulfide, age, and complaint duration values between groups

Variable	Mean ± SD	Median (Min-Max)	F/χ^2	p
WBC*			59.491	< 0.001*
Grade I	9.15 ± 2.89	9.25 (3.30-14.50)		
Grade II	14.76 ± 3.92	14.05 (5.10-21.30)		
Grade III	15.98 ± 5.28	18.00 (3.80-22.20)		
Control	6.77 ± 1.22	6.45 (5.10-9.30)		
CRP**			91.955	< 0.001*
Grade I	8.16 ± 5.44	7.12 (0.28-21)		
Grade II	49.92 ± 34.73	38.50 (19.56-214)		
Grade III	181.27 ± 73.20	186.50 (51.29-56)		
Control	-	-		
Albumin**			65.033	< 0.001*
Grade I	3.09 ± 0.63	3.20 (0.73-3.87)		
Grade II	2.84 ± 0.77	3.00 (0.36-4.27)		
Grade III	2.36 ± 0.82	2.48 (0.53-3.52)		
Control	3.62 ± 0.27	3.61 (2.56-4.13)		
IMA**			31.470	< 0.001*
Grade I	0.81 ± 0.23	0.80 (0.45-1.53)		
Grade II	0.86 ± 0.22	0.83 (0.50-1.48)		
Grade III	1.14 ± 1.43	0.88 (0.42-8.46)		
Control	0.61 ± 0.19	0.59 (0.28-1.17)		
Index 1**			52.543	< 0.001*
Grade I	6.89 ± 5.21	5.26 (0.53-20.85)		
Grade II	10.55 ± 7.57	8.02 (1.08-34.21)		
Grade III	15.35 ± 11.22	10.40 (5.71-42.80)		
Control	4.39 ± 1.21	4.31 (2.18-9.73)		
Index 2**			52.553	< 0.001*
Grade I	5.73 ± 3.71	4.76 (0.53-14.71)		
Grade II	8.16 ± 4.62	6.91 (1.06-20.31)		
Grade III	10.79 ± 5.81	8.61 (5.12-23.06)		
Control	4.01 ± 1.03	3.97 (2.09-8.15)		
Index 3**			52.517	< 0.001*
Grade I	88.55 ± 7.42	90.49 (70.57-98.94)		
Grade II	83.68 ± 9.24	86.18 (59.38-97.88)		
Grade III	78.42 ± 11.62	82.79 (53.88-89.76)		
Control	91.98 ± 2.06	92.07 (83.71-95.83)		
INR**			10.900	0.004*
Grade I	1.21 ± 0.88	1.06 (0.91-690)		
Grade II	1.30 ± 0.95	1.14 (0.90-6.60)		
Grade III	1.34 ± 0.38	1.19 (0.98-2.20)		
Control	-	-		
Native thiol*			28.635	< 0.001*
Grade I	291.04 ± 74.19	295.97 (139.20-456.76)		
Grade II	253.89 ± 85.50	232.88 (85.38-440.60)		
Grade III	215.50 ± 83.45	216.81 (92.10-392.23)		
Control	369.07 ± 55.15	367.35 (229.58-527.63)		
Total thiol*			26.276	< 0.001*
Grade I	325.39 ± 67.05	318.10 (192.24-487.24)		
Grade II	296.72 ± 74.65	279.62 (143.79-459.18)		
Grade III	266.99 ± 77.28	271.75 (152.50-443.42)		
Control	400.82 ± 56.55	400.33 (261.84-566.58)		
Disulfide**			34.365	< 0.001*
Grade I	17.17 ± 9.10	15.77 (1.66-46.75)		
Grade II	21.42 ± 7.94	19.69 (4.46-38.89)		
Grade III	25.74 ± 9.15	21.46 (13.15-45.83)		
Control	15.88 ± 4.13	15.46 (8.75-36.60)		
Age*			24.829	< 0.001*
Grade I	54.98 ± 17.41	57.50 (22.00-86.00)		
Grade II	64.12 ± 17.79	66.00 (18.00-92.00)		
Grade III	71.60 ± 16.89	74.50 (24.00-91.00)		
Control	41.40 ± 10.46	42.00 (26.00-62.00)		
Symptoms (days)**			73.328	< 0.001*
Grade I	1.73 ± 0.45	2.00 (1.00-2.00)		
Grade II	5.06 ± 3.37	4.00 (1.00-20.00)		
Grade III	6.20 ± 2.34	5.50 (3.00-12.00)		

*Analysis of variance analysis was performed because the data met normal distribution assumptions.
**Kruskal–Wallis test was performed because the data did not meet normal distribution assumptions.
WBC: white blood cells; CRP: C-reactive protein; IMA: ischemia-modified albumin; INR: international normalized ratio.

cutoff value of 331.38, achieving 78.7% sensitivity and 83.3% specificity (95% confidence interval [CI]: 0.798-0.918). TT had an AUC of 0.849 ($p < 0.001$), a cutoff value of 363.58, a sensitivity of 79.6%, and a specificity of 81.0% (CI: 0.786-0.913). The index 3 ratio had an AUC of 0.784 ($p < 0.001$), with an optimal cutoff value of 91.20, 77.8% sensitivity, and 78.6% specificity (CI: 0.710-0.857) (Fig. 2).

Disulfide showed an AUC of 0.696 ($p < 0.001$), a cutoff value of 16.57, 65.7% sensitivity, and 66.7% specificity (CI: 0.612-0.779).

In addition, the D/NT ratio (Index 1) and D/TT ratio (Index 2) showed AUCs of 0.784 ($p < 0.001$) and 0.665 ($p < 0.001$), respectively, with optimal cutoff values of 4.82 and 4.395. Both ratios had 77.8% sensitivity and 78.6% specificity (CI: 0.711-0.858 and 0.710-0.857).

Finally, IMA showed an AUC of 0.791 ($p < 0.001$), a cutoff value of 0.655, and a sensitivity of 75.0% and specificity of 71.4% (CI: 0.709-0.872). The present findings suggest that the oxidative stress markers used in this study can be used as a laboratory test in addition to radiologic tests to distinguish between patients with acute cholecystitis and healthy people (Table 4 and Fig. 3).

Discussion

Significant changes in inflammatory and oxidative stress indicators were observed in patients with

Table 3. Correlation coefficients between age, duration of symptoms, CRP, WBC, albumin, IMA, Index 1, Index 2, Index 3, native thiol, total thiol, and disulfide in the patient group

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Age	-												
r	-												
p	-												
2. Symptoms (days)		-											
r	0.380	-											
p	0.000	-											
3. CRP			-										
r	0.355	0.776	-										
p	0.000	0.000	-										
4. WBC				-									
r	0.096	0.358	0.604	-									
p	0.325	0.000	0.000	-									
5. Albumin					-								
r	−0.321	−0.335	−0.358	−0.194	-								
p	0.001	0.000	0.000	0.044	-								
6. IMA						-							
r	0.157	0.119	0.106	−0.092	−0.032	-							
p	0.105	0.220	0.274	0.342	0.743	-							
7. Index 1							-						
r	0.320	0.351	0.391	0.356	−0.489	0.055	-						
p	0.001	0.000	0.000	0.000	0.000	0.569	-						
8. Index 2								-					
r	0.319	0.351	0.391	0.356	−0.489	0.056	1.000	-					
p	0.001	0.000	0.000	0.000	0.000	0.567	0.000	-					
9. Index 3				−0.355					-				
r	−0.320	−0.351	−0.390	0.000	−0.489	−0.055	−1.000	−1.000	-				
p	0.001	0.000	0.000		0.000	0.571	0.000	0.000	-				
10. Stage										-			
r	0.399	0.809	0.927	0.596	−0.396	0.082	0.441	0.441	−0.440	-			
p	0.000	0.000	0.000	0.000	0.000	0.399	0.000	0.000	0.000	-			
11. Native thiol											-		
r	−0.346	−0.266	−0.318	−0.247	0.613	−0.097	−0.858	−0.858	0.858	−0.355	-		
p	0.000	0.005	0.001	0.010	0.000	0.318	0.000	0.000	0.000	0.000	-		
12. Total thiol												-	
r	−0.341	−0.226	−0.275	−0.198	0.629	−0.109	−0.764	−0.764	0.764	−0.310	0.982	-	
p	0.000	0.019	0.004	0.040	0.000	0.261	0.000	0.000	0.000	0.001	0.000	-	
13. Disulfide													-
r	0.236	0.321	0.354	0.381	−0.324	−0.011	0.930	0.930	−0.930	0.398	−0.645	−0.513	-
p	0.014	0.001	0.000	0.000	0.001	0.914	0.000	0.000	0.000	0.000	0.000	0.000	-

WBC: white blood cells; CRP: C-reactive protein; IMA: ischemia-modified albumin; INR: international normalized ratio.

acute cholecystitis as the stage progressed. In particular, the increase in inflammatory indicators such as WBC, CRP, disulfide, and IMA and the decrease in antioxidant parameters such as albumin, NT, and TT are directly proportional to the stage of the disease.

Complex acute cholecystitis can lead to sepsis from gallbladder inflammation[2]. In 2018, the Tokyo Acute Cholecystitis Treatment Guideline was finalized after several updates to the timing of cholecystectomy and the selection of intensive care patients. WBC is the only laboratory marker used in disease staging by the Tokyo criteria[4].

WBC rises as a defense. Previous research describes acute cholecystitis WBC elevation up to 77%[10]. Our study found a statistically significant increase in all

Table 4. ROC analysis of oxidative stress parameters and confidence ıntervals

Test name	Zone	p	Cut off value	Sensitivity (%)	Specifity (%)	Confidence interval
Native thiol	0.858	< 0.001*	331.38	78.7	83.3	(0.798-0.918)
Total thiol	0.849	< 0.001*	363.58	79.6	81.0	(0.786-0.913)
(index 3)	0.784	< 0.001*	91.20	77.8	78.6	(0.710-0.857)
Disulfide	0.696	< 0.001*	16.57	65.7	66.7	(0.612-0.779)
(index 1)	0.784	< 0.001*	4.82	77.8	78.6	(0.711-0.858)
(index 2)	0.665	< 0.001*	4.395	77.8	78.6	(0.710-0.857)
IMA	0.791	< 0.001*	0.655	75	71.4	(0.709-0.872)

*For marked variables, only the numbers and percentages in the patient group are given. It does not include the control group.
ROC: receiver operating characteristic; IMA: ischemia-modified albumin.

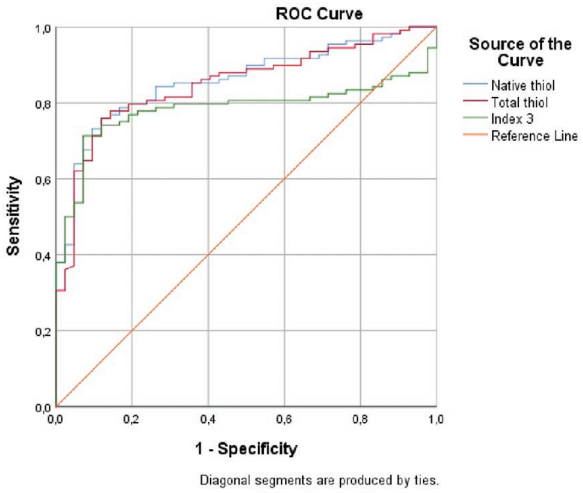

Figure 2. *Performance of native thiol, total thiol, and index 3 in differentiating acute cholecystitis form healthy controls.*

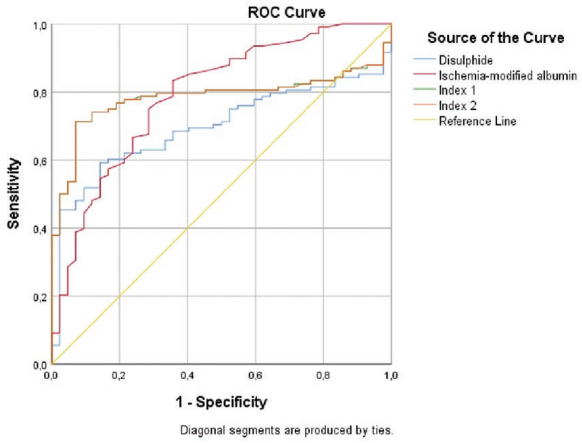

Figure 3. *Performance of acute ischemia-modified albumiin, disulphide, index 1, and index 2 in differentiating acute cholecystitis from healthy controls.*

patient groups compared to healthy individuals. Stages II and III had significantly more WBCs than Stage I, reflecting the progression of inflammation. Fagan et al. found that GC patients had significantly higher WBC counts than uncomplicated patients. Male gender and WBC counts above 15.000 microliter were risk factors for GC[11]. Our study found 14.700 and 18.000 microliter leukocyte counts in Stages II and III patients, consistent with previous research.

Acute-phase reactant C-reactive protein helps the body respond to trauma and inflammation. It should rise with inflammation. If pre-operative plasma levels were higher than post-operative CRP and WBC levels, post-operative complications, and conversion to open surgery increased[12]. Kabul Gurbulak et al. analyzed 682 patients retrospectively. They found that CRP levels rose as disease complexity increased in Tokyo 2013 guideline patients[13]. Despite the lack of studies, our study found that CRP levels increased significantly between groups as disease severity increased in all groups.

In mammals, albumin is the most common plasma protein. Oxidants may target plasma protein sulfhydryl groups. The unique free sulfhydryl group forms a sulfenic group in albumin for oxidation[14]. Albumin has many intracellular functions, but it is a negative acute phase reactant that participates in inflammation and decreases serum levels. High CRP and low albumin levels increase inflammatory disease mortality, especially in elderly patients[15]. Kaysen et al. found a negative correlation between CRP and serum albumin in hemodialysis patients[16]. All patient groups had significantly lower albumin levels

than the control group, and as disease severity increased, albumin levels decreased. Symptom duration increased with age, CRP, and disulfide.

This is the first study to compare thiol/disulfide homeostasis and IMA levels, which are thought to be markers of oxidative stress in acute cholecystitis patients, to inflammatory markers and disease grades according to the Tokyo 2018 guidelines.

IMA was initially approved by the Food and Drug Administration (FDA) for use in early prediction of myocardial ischemia in cardiac patients[17]. Later, studies used IMA to predict ischemia and necrosis in many diseases. Sinha et al. discovered that IMA preceded myocardial ischemia[18]. IMA can predict ischemia or severe disease in many diseases, including skeletal muscle ischemia, acute appendicitis, and mesenteric ischemia[19,20]. Sahin et al. found that acute pancreatitis severity increased IMA and corrected IMA levels[21]. Our study found a significant difference in IMA values between acute cholecystitis patients and healthy controls. The median ABSU values of all groups in our study were above 0.400 and 0.400 ABSU is considered as the threshold value for IMA and values above this value suggest ischemia[9]. The difference between Stage I and Stage III patients was statistically significant. In all stage groups, the median IMA level in patients was higher than in controls. Statistics showed that Stages II and III were significantly higher than the control group. Based on these results, IMA may be a marker for Grade II-III acute cholecystitis.

Thiols are sulfhydryl sulfide and hydrogen groups that participate in intracellular and extracellular antioxidant reactions. Plasma thiols are albumin or lower molecular weight cysteine-like protein thiols. Due to inflammation, free radicals increase, and the antioxidant system neutralizes them. Oxidative stress is caused by insufficient antioxidant defense during inflammation, but after antioxidant thiols become active, NTs decrease, and disulfide is expected to increase in response to free radicals[22]. In bacterial tonsillitis, Familial Mediterranean fever (FMF), tumors, and acute pancreatitis, serum thiol/disulfide values were in the disulfide direction[23]. FMF patients had higher disulfide and lower native and TT values than healthy people[24]. Bacterial tonsillitis patients had higher index 1 and index 2 ratios than controls[25]. Another study found that gallbladder perforation patients had higher median WBC, neutrophil count, disulfide, and IMA values than gallstone patients[26]. Researchers found that the healthy control group had significantly higher levels of native and TT ($p < 0.001$) compared to all other groups of patients with acute cholecystitis. The duration of symptoms, CRP value, disease severity, and disulfide were positively correlated, while total and NT levels were negatively correlated.

These findings showed that oxidative stress exposure increases with disease severity, inflammatory laboratory parameters, and antioxidants drop and oxidants rise. Since oxidative stress shifts the oxidant-antioxidant balance to the oxidant direction in inflammatory diseases, antioxidants may help treat them[27].

Conclusion

This study demonstrated that thiol-disulfide homeostasis and IMA can be used as a laboratory marker supporting radiologic and clinical parameters to differentiate between complex patient groups with severe acute cholecystitis and low-grade patient groups.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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